

# CFA

## Complete Freund's Adjuvant

Catalog # vac-cfa-10, vac-cfa-60

For research use only. Not for use in humans.

Version # 15J21-MM

## PRODUCT INFORMATION

### Content:

CFA (Complete Freund's Adjuvant) is provided as a ready-to-use, pale yellow liquid. CFA is available in two quantities:

- 10 ml: cat. code # vac-cfa-10
- 6 x 10 ml: cat. code # vac-cfa-60

Each 10 ml CFA vial contains 10 mg heat killed dried *Mycobacterium tuberculosis* (strain H37Ra), 1.5 ml Mannide Monooleate and 8.5 ml Paraffin oil. As CFA contains particulate matter, it may appear slightly hazy.

### Storage and stability

- CFA is shipped at room temperature and should be stored at 4°C.
- CFA is stable for 2 years when properly stored. **DO NOT FREEZE.**

### Quality control

- CFA is VacciGrade™ (preclinical grade). It is prepared under strict aseptic conditions and is tested for the presence of endotoxins. CFA is guaranteed sterile and its endotoxin level is <1 EU/ml.

- Adjuvanticity of CFA was evaluated by assessing the levels of total mouse IgGs (mIgGs) and the mIgG1 and mIgG2 isotypes after an initial subcutaneous injection of EndoFit™ Ovalbumin/CFA (1:1, v/v) in mice. To minimize side-effects, EndoFit™ Ovalbumin/IFA (1:1, v/v) was used for a subcutaneous boost injection. Results were compared to mice which received the antigen alone.

## DESCRIPTION

Complete Freund's Adjuvant (CFA) consists of heat-killed *Mycobacterium tuberculosis* in non-metabolizable oils (paraffin oil and mannide monooleate)<sup>1</sup>. CFA contains trehalose 6,6' dimycolate (TDM), which stimulates Mincle<sup>2</sup>. Additionally, CFA has ligands for TLR2, TLR4, and TLR9. Injection of antigen in CFA induces a Th1-dominated response when compared to injection in Incomplete Freund's Adjuvant (IFA), which lacks mycobacterial components and induces a Th2-dominated response. An essential component of CFA-mediated response is an intense inflammatory reaction at the site of antigen deposition. CFA is utilized to produce water-in-oil emulsions of antigens. For most applications, CFA is usually only necessary for the initial immunization, while IFA is the adjuvant of choice for subsequent immunizations.

Typical results obtained with CFA are shown in figure 1.

1. Lindblad EB., 2000. Freund's Adjuvants. In: Vaccine adjuvants: Preparation Methods and Research Protocols. Humana Press. Totowa, NJ. 2. Coffman RL. et al., 2010. Vaccine adjuvants: putting innate immunity to work. Immunity. 33(4):492-503.

## METHODS

### Preparation of antigen-CFA emulsions

Antigens are preferentially diluted in saline or phosphate buffers. The amount of protein or conjugated peptide used for the primary immunization can be adjusted depending upon availability and immunogenicity of the antigen. For example, mice can be injected subcutaneously (s.c.) with 1 to 10 µg of endotoxin-free ovalbumin (cat. code #vac-pova).

1. Mix equal volumes of antigen and CFA using a 21 gauge needle and syringe. A white emulsion will form immediately.
2. Mix vigorously for several minutes to form an emulsion with maximum stability.

*Note: A stable emulsion is essential to produce high titers of antibodies.*

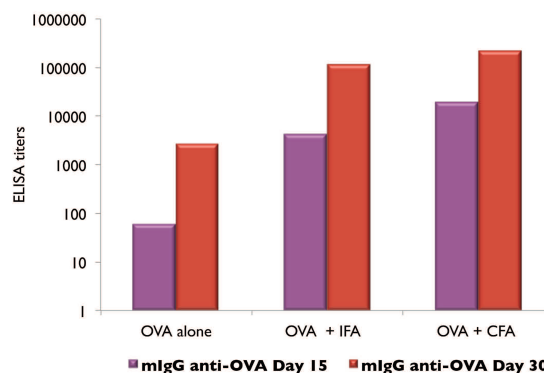
3. Vortex just before use.

The volume of injection depends of the site of administration.

*Note: To avoid anaphylaxis, do not use adjuvants for intravenous injection.*

Species	Max. volume	Injection Site
Mice, hamsters	100 µl	subcutaneous (s.c.)
Mice, hamsters	50 µl	intramuscular (i.m.)
Guinea pigs	200 µl	s.c. or i.m.
Rats	200 µl	s.c. or i.m.
Rabbits	250 µl	s.c. or i.m.

Recommended maximum volumes for injection of antigen/adjuvant mixtures per site of injection for laboratory animals. (Reference: Lindblad EB., 2000. Freund's Adjuvants. In: Vaccine adjuvants: Preparation Methods and Research Protocols. Humana Press. Totowa, NJ).



**Figure 1.** Anti-Ova mIgG levels at 15 and 30 days after the initial immunization. On Day 1, mice were immunized s.c. with 1 µg of EndoFit™ Ovalbumin alone, with IFA (1:1, v/v) or CFA (1:1, v/v) in a final volume of 100 µl. On Day 15, mice treated with IFA or CFA were immunized with 1 µg of EndoFit™ Ovalbumin with IFA (1:1, v/v) in a final volume of 100 µl. Serum anti-OVA mIgG was monitored by ELISA.

## TECHNICAL SUPPORT

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## RELATED PRODUCTS

Product	Description	Catalog Code
<b>Adjuvants</b>		
AddaVax™	Squalene-Oil-in-water	vac-adx-10
Alhydrogel 2%	Aluminium hydroxide gel	vac-alu-250
IFA	Incomplete Freund's adjuvant	vac-ifa-10
Poly(I:C) VacciGrade™	TLR3 agonist	vac-pic
MPLA VacciGrade™	TLR4 agonist	vac-mpla
Flagellin FliC VacciGrade™	TLR5 agonist	vac-fla
Gardiquimod VacciGrade™	TLR7 agonist	vac-gdq
Imiquimod VacciGrade™	TLR7 agonist	vac-imq
R848 VacciGrade™	TLR7/8 agonist	vac-r848
ODN 1826 VacciGrade™	murine TLR9 agonist	vac-1826-1
ODN 2006 VacciGrade™	human TLR9 agonist	vac-2006-1
N-glycolyl-MDP VacciGrade™	NOD2 agonist	vac-gmdp
<b>OVA Antigens</b>		
EndoFit™ Ovalbumin	For <i>in vivo</i> use; endotoxin level <1EU/mg	vac-pova
Ovalbumin	For detection; Western, ELISA	vac-stova
Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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